



Ballast Water Detecting Lab of Shanghai Ocean University



No. SHOU-PACT-2014002

# Testing Report

Product Name: Ballast Water (water quality, 10~50  $\mu\text{m}$ ,  $\geq 50 \mu\text{m}$  and bacteria)

Applicant: PACT Environmental Protection Technology co., LTD

Test Category: Sampling Test

Issued Date: April 30, 2014

**Ballast Water Detecting Lab (BWDL) of Shanghai Ocean University****Announcement**

1. The report is considered to be invalid in case of no official stamp of “Ballast Water Detecting Lab of Shanghai Ocean University” on it;
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3. The report is considered to be invalid without signatures of the reviewer and approver;
4. The report is considered to be invalid once it is altered;
5. Any questions on the report should be raised to the testing organization within 15 days since the date you receive the report and the overdue is inadmissible.
6. Test results only indicate this sampling.

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## Ballast Water Detecting Lab (BWDL) of Shanghai Ocean University

## Testing Report of Organisms

No. SHOU-PACT-2014002

Sample name	Ballast water	Applicant	PACT Environmental Protection Technology co., LTD
Test category	Sampling test	Address of the applicant	5/F, No.700 Luban Road, Shanghai 200023, China
No. of samples	270	Sample ID number	SHOU-BWDL- PACT -013A1~A18 SHOU-BWDL-PACT-013B1~B18 SHOU-BWDL-PACT-013C1~C18 5 batches, 18 samples for each batch
Sample	Liquid	Sampling personnel	LiangLiu, Jianfei Jiang, Zhangwei Guo
Sample delivery date	2013.09.15~2013.09.17 2013.10.11~2013.10.13 2013.10.27~2013.10.29 2013.10.29~2013.10.31 2014.04.03~2014.04.05	Test date	2013.09~2014.04
		Sample site	Hong Tai 158
Test items	Reference standards		
10~50 $\mu\text{m}$	GB 17378.7-2007/5.3.2.3 concentration counting method		
$\geq 50 \mu\text{m}$	GB 17378.7-2007/5.3.3.3 individual counting method		
Heterotrophic bacteria; <i>Escherichia coli</i> ; <i>Vibrio cholera</i> ; intestinal <i>Enterococci</i>	Plate counting method in the Specification for Marine Monitoring-Part 7: Ecological survey for offshore pollution and biological monitoring (GB17378.7-2007/10.1); Filter membrane method in the specification for marine monitoring-Part 7: Ecological survey for offshore pollution and biological monitoring (GB 17378.7-2007/9.2); Diagnosis Standard for <i>Vibrio cholera</i> WS 289-2008; Water Quality - Detection and Enumeration of Intestinal <i>Enterococci</i> in Surface and waste water - Membrane Filtration Method ISO 7899-2:2000.		

Written by: Yuan Lin Checked by: Wang Bing Approved by: Xue Junzeng



## Ballast Water Detecting Lab (BWDL) of Shanghai Ocean University

## Testing Report of Water Qualities

No. SHOU-PACT-2014002

Sample name	Ballast water	Applicant	PACT Environmental Protection Technology co., LTD	
Test category	Sampling test	Address of the applicant	5/F, No.700 Luban Road, Shanghai 200023, China	
No. of samples	90	Sampling personnel	Huixian Wu, Qiong Wang, Lin Yuan, Liang Liu	
Sample	Seawater	Test date	2013.09~2014.04	
Sample delivery date	2013.09.15~2013.09.17 2013.10.11~2013.10.13 2013.10.27~2013.10.29 2013.10.29~2013.10.31 2014.04.03~2014.04.05		Sample site	Hong Tai 158
Test items	Reference standards			
Temperature Salinity TSS POC	Marine Monitoring—Part 4: Seawater analysis (GB17378.4-2007) 25.1 Surface thermometer method, 27 Gravimetric Method, and 29.1 Salimeter Method. Spectrophotometric Method in the Gulf Ecosystem Observation Method, China Environmental Science Press, 2005.			
Sample number	SHOU-BWDL-PACT-013D1~D18; SHOU-BWDL-PACT-014D1~D18; SHOU-BWDL-PACT-015D1~D18; SHOU-BWDL-PACT-016D1~D18; SHOU-BWDL-PACT-017D1~D18; 5 batches, 18 samples for each batch			

Written by: Yuan Lin    Checked by: Liu Liang    Approved by: Xue Jirang

## Conclusions

203-day shipboard tests for five batches were conducted on "Hongtai 158", using PACT Marine<sup>TM</sup> Ballast Water Management System (BWMS) (with a capacity of 300 m<sup>3</sup>/h), developed by Yixing PACT Environmental Protection Technology Co., Ltd. The test periods met requirement of IMO G8 Guidelines. The test results were compared to IMO G8 requirement and D-2 standard and the following conclusions were drawn:

1. For test No. 013, densities of organisms  $\geq 50 \mu\text{m}$  in discharged water of PACT Marine<sup>TM</sup> BWMS (with a capacity of 300 m<sup>3</sup>/h) were higher than the requirement of IMO G8, so it was an invalid cycle. Densities of organisms  $\geq 50 \mu\text{m}$  in influent water for the subsequent four cycle tests (i.e., test No. 014-017) were all within  $2.32 \times 10^3 \sim 1.82 \times 10^4 \text{ ind./m}^3$  with average density of  $8.55 \times 10^3 \text{ ind./m}^3$ , which met the IMO G8 requirement. Average densities of organisms  $\geq 50 \mu\text{m}$  in discharged water from treated tanks for four valid tests (i.e., test No. 014-017), were 6.67, 6.11, 1.89 and 3.22 ind./m<sup>3</sup>, respectively. Average densities of organisms  $\geq 50 \mu\text{m}$  in discharged water from control tanks for four valid tests (i.e., test No. 014-017), were  $2.00 \times 10^3$ ,  $1.09 \times 10^4$ ,  $6.88 \times 10^3$  and  $1.34 \times 10^3 \text{ ind./m}^3$ , respectively. Densities of organisms  $\geq 50 \mu\text{m}$  in discharged water both from treated tanks and control tanks were met D-2 discharge standard and IMO G8 requirement.
2. Densities of organisms  $\geq 10 \sim 50 \mu\text{m}$  in influent water for five test cycles were all within  $1.36 \times 10^2 \sim 5.05 \times 10^2 \text{ cells/mL}$  with an average density of  $3.07 \times 10^2 \text{ cells/mL}$ , which met the requirement of IMO G8. Average densities of living organisms  $\geq 10 \sim 50 \mu\text{m}$  in discharged water from treated tanks were 2.78, 0.29, 0.04, 0.02 and 0.09 cells/mL, respectively. Average densities of living organisms  $\geq 10 \sim 50 \mu\text{m}$  in discharge water from control tanks were  $3.28 \times 10^2$ , 12.67, 43.33, 15.00 and  $1.15 \times 10^2 \text{ cells/mL}$ , respectively. Organisms  $\geq 10 \sim 50 \mu\text{m}$  in discharged water from treated tanks and control tanks met D-2 discharge standard and IMO G8 requirement, respectively.
3. Total number of heterotrophic bacteria in influent water for the five tests (i.e., test No. 013-017) were  $1.1 \times 10^4$ ,  $2.1 \times 10^4$ ,  $7.3 \times 10^4$ ,  $2.7 \times 10^4$  and  $1.0 \times 10^4 \text{ CFU/mL}$ , respectively. Average concentrations of heterotrophic bacteria in discharged water from treated tank for the five tests (i.e., test No. 013-017) were 333.9, 297.7, 271.8, 527.8 and 85.7 CFU/mL, respectively. The average concentrations of heterotrophic bacteria in discharged water from control tanks were  $2.4 \times 10^4$ ,  $4.6 \times 10^4$ ,  $3.8 \times 10^4$ ,  $6.5 \times 10^4$  and  $6.5 \times 10^3 \text{ CFU/mL}$ , respectively.
4. Average concentrations of *Escherichia coli* in influent water for the five tests (i.e., test No. 013-017) were within  $1.4 \times 10^4 \sim 4.3 \times 10^5 \text{ CFU/100 mL}$ . Average concentrations of *Escherichia coli* in discharged water from treated tanks for the five tests (i.e., test No. 013-017) were

116.1, 99.8, 61.7, 35.7 and 46.0 CFU/100 mL, respectively. The average concentrations of *Escherichia coli* in discharge water from control tanks were  $1.4 \times 10^5$ ,  $5.4 \times 10^4$ ,  $4.9 \times 10^5$ ,  $8.2 \times 10^4$  and  $6.4 \times 10^3$  CFU/100 mL, respectively. These results met IMO G8 requirement and D-2 discharge standard.

5. No intestinal *enterococci* and *vibrio cholerae* were detected in discharged water for the five cycle tests (i.e., test No. 013-017).

In summary, except test No. 013, the other four cycle tests (i.e., test No. 014-017) were consecutive and effective, and their test procedures and results met IMO G8 requirement and D-2 standard.

Note: The reason for unqualified density of organisms  $\geq 50 \mu\text{m}$  in discharged water of test No. 013 was attributed to the fact that the ballast tank on Hongtai 158 was not cleaned up before the installation of PACT Marine™ BWMS ( with a capacity of  $300 \text{ m}^3/\text{h}$  ) .



## Preface

The shipboard testing conducted from September 15, 2013 to April 5, 2014 on Hong Tai 158. The first cycle (013) test covered the shipping line from the East China Sea near the Yellow Sea (influent water) to the Northern Yellow Sea (discharge water). BWDL conducted the test as a contract assignment for PACT Environmental Protection Technology co., LTD.

During planning and conducting of the test, Le Zhang were the leaders representative of PACT Environmental Protection Technology co., LTD. Huixian Wu, Lin Yuan, Qiong Wang, Liang Liu were the main representatives for BWDL. Other representatives of PACT Environmental Protection Technology co., LTD and BWDL were also involved in this project.

We want to thank PACT Environmental Protection Technology co., LTD for choosing BWDL as a main partner for the test and the verification of the PACT Marine<sup>TM</sup> BWMS (with a capacity of 300 m<sup>3</sup>/h) . We also appreciate the contribution of all the staff involved in this project.

This version of the report describes the land-based type approval tests, according to IMO's Guidelines for approval of ballast water management systems.

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## 1. Background

With the rapid development of the world-trade and global tourism, the demand for free trade is growing and the marine shipping industry is exuberant and occupies 60% share of world trade. To assure the safety of sailing ships, it is necessary to add some ballast to keep the ship in an appropriate stable and floating status. Since 1980s, it is common to use water as ballast, the it is the so called ballast water.

Ballast water causes a spread of species from one water region to another; in fact ballast water in a ship contains harmful aquatic organisms or pathogens that, if discharged to the waters of another world region, it will endanger the local ecology, economy and human health. The effects will last for a long time. Once the aquatic organisms invade and inhibit in the local waters, they will reproduce in an uncontrollable manner and destroy the eco-system of local species, lead to mass propagation of harmful parasite and pathogen and even extinguish the local species.

The test and management of ballast water is getting more and more important as the ocean pollution is getting worse due to the ships ballast water discharges. Aiming to prevent the potentially devastating effects of the spread of harmful aquatic organisms and pathogens carried by ship's ballast water from one region to another, IMO proposed and approved the *International Convention for the Control and Management of the Ship's Ballast Water and Sediments* (hereinafter BWM Convention) in 2004. The BWM Convention requires the following points:

- average density of organisms greater or equal than 50 micrometers in minimum diameter in the replicate sample, has to be less than 10 viable organisms per cubic meter;
- average density of organisms smaller than 50 micrometers but bigger or equal than 10 micrometers in minimum diameter in the replicate samples has to be less than 10 viable organisms per milliliter;
- average density of *Vibrio cholerae* (serotypes O1 and O139) has to be less than 1 cfu per 100 milliliters, or less than 1 cfu per 1 gram (wet weight) of zooplankton sample;
- average density of *E. coli* in the replicate samples has to be less than 250 cfu per 100 milliliters;
- average density of intestinal *enterococci* in the replicate samples has to be less than 100 cfu per 100 milliliters.

According to the Guidelines G8 of the IMO, the approval of ballast water management systems

are required to perform series of testing on board, in order to assess whether ballast water management systems meets the standard as set out in regulation D-2 of the BWM Convention.

## 2. Introduction

### 2.1 Ballast Water Detecting Lab of Shanghai Ocean University

Ballast Water Detecting Lab (BWDL) of Shanghai Ocean University was founded in September 2008. There are twenty-two persons in the lab, among which four persons are engineers and scientists with high professional title. The lab consists of sample acceptance room, hydrochemistry room, microorganism testing room, microscope room, balance room and sample storage room. The lab is dedicated to the study of the harbor ecology and invasion ecology: the main focuses of the ecology research are the study of the plankton in harbor area, the ship ballast water and the microorganisms in ocean environment.

The lab is equipped with all the typologies of instruments and apparatus, such as BOD<sub>5</sub> analyzer, TOC analyzer, spectrophotometer, stereoscopic microscope, conductivity gauge, turbidimeter for water micro-organism test environmental parameters detection and plankton test.

The related staff is asked to be trained before they conduct the testing task. The six doctors and sixteen masters are all specialized in the parameter field. By now, BWDL is able to test five organism indicators and ten water quality parameters in accordance with the ballast water discharging standards regulated in the International Convention for the Control and Management of the Ship's Ballast Water and Sediments:

1. viable organisms greater than or equal to 50  $\mu\text{m}$  in minimum dimension;
2. viable organisms less than 50  $\mu\text{m}$  and greater than or equal to 10  $\mu\text{m}$  in minimum dimension;
3. toxicogenic *Vibrio cholera* (serotypes O1 and O139);
4. *Escherichia coli*;
5. Intestinal *Enterococci*;
6. heterotrophic bacteria;
7. total residual oxidants (TRO);
8. dissolved oxygen (DO);
9. total suspended solids (TSS);



10. turbidity;
11. dissolved organic carbon (DOC);
12. particulate organic carbon (POC);
13. pH;
14. salinity;
15. temperature.

Being realistic and creative, BWDL aims to build a competent and famous lab which is specialized in the testing of ship's ballast water in China.

## **2.2 PACT Marine<sup>TM</sup> BWMS with a capacity of 300 m<sup>3</sup>/h**

PACT Marine<sup>TM</sup> BWMS with a capacity of 300 m<sup>3</sup>/h is developed by PACT Environmental Protection Technology co., LTD that is a multinational engineering company specializing in custom design, manufacturing, contracting and BOT/BOOT/BOO of water treatment, wastewater treatment and water desalination. A great majority of PACT clients are global/fortune 500 companies, leaders in their industry.

PACT products are the result of years of interaction between PACT engineers, end-users and contractors. PACT projects custom built to suit the individual application and the local conditions.

PACT engineers have a broad range of expertise in physical, chemical, biological and membrane processes for water and wastewater treatment. In every discipline, PACT provides numerous process choices to solve complex treatment problems effectively and economically.

### 3. Test Procedures

#### 3.1 Sampling points arrangement

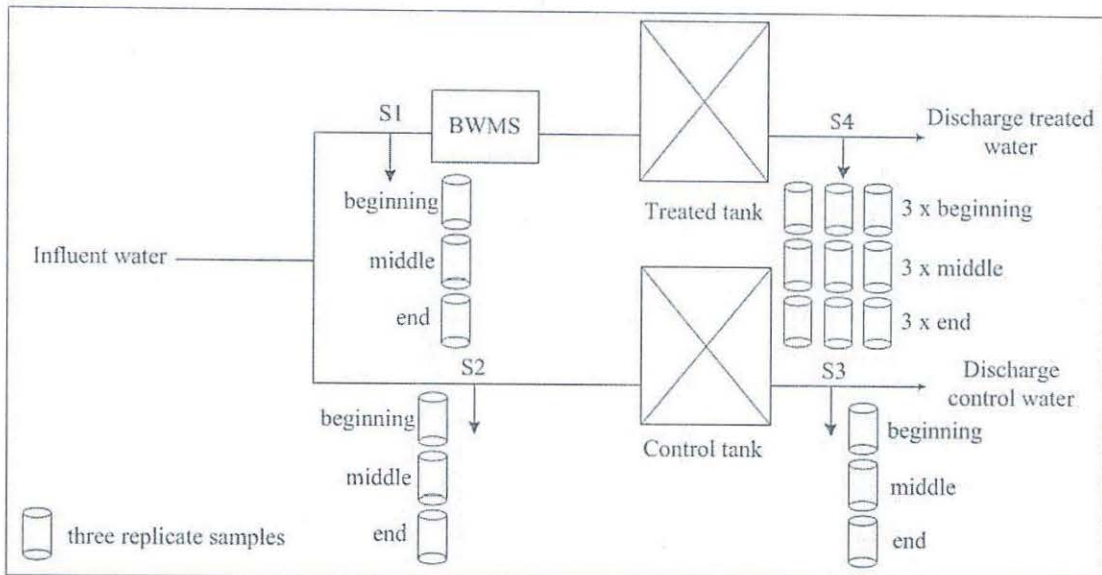
In a whole test cycle, the influent water samples are taken during ballast water uptake. Sampling ports are located as close as practicable to the BWMS prior to treatment to determine concentrations of living organisms upon uptake.

The discharge samples from both the treated tank and the control tank are taken when discharging. Sampling ports are located as close as practicable to the BWMS overboard outlet prior to the discharge point to determine concentrations of living organisms prior to discharge.

The sampling is performed by qualify personnel (Figure 1). The arrangement of the sampling point is shown in Figure 2. S1 will be three replicate samples of influent water, collected over the period of uptake on the treatment track (e.g., beginning, middle, end); S2 will be three replicate samples of influent water, collected over the period of uptake on the control track (e.g., beginning, middle, end); S3 will be three replicate samples of discharge control water, collected over the period of discharge (e.g., beginning, middle, end); and S4 will be three replicate samples of discharge treated water collected at each of three times during the period of discharge (e.g., 3 × beginning, 3 × middle, 3 × end).



Figure 1. Field sampling on Hongtai 158



**Figure 2. Arrangement of the sampling points** (S1, influent water on the treatment track; S2, influent water on the control track; S3, control water at discharge; S4, treated water at discharge)

## 3.2 Sampling

### 3.2.1 Sample collection

First microbiological samples are collected and then samples for water quality. Table 1 summarizes which sampling equipment is used to collect samples for the individual parameters.

**Table 1. Equipment and containers used for sampling and sampled volume for the individual parameters**

Parameter	Sampling equipment	Sample container	Collected volume S1, S2, S3	Collected volume S4
Organisms $\geq 50 \mu\text{m}$	Directly	Clean HDPE bottle	$3 \text{ m}^3$ ( $3 \times 1 \text{ m}^3$ )	$9 \text{ m}^3$ ( $3 \times 3 \times 1 \text{ m}^3$ )
Organisms $\geq 10 \sim 50 \mu\text{m}$	Directly	Clean HDPE bottle	$30 \text{ L}$ ( $3 \times 10 \text{ L}$ )	$90 \text{ L}$ ( $3 \times 3 \times 10 \text{ L}$ )
<i>E. coli</i>	Directly	Clean glass bottle	$3000 \text{ mL}$ ( $3 \times 1000 \text{ mL}$ )	$9000 \text{ mL}$ ( $3 \times 3 \times 1000 \text{ mL}$ )
Intestinal <i>Enterococci</i>	Directly	Clean glass bottle		
Heterotrophic bacteria and <i>Vibrio cholerae</i>	Directly	Clean glass bottle		
Salinity	Directly	Clean HDPE bottle	$6 \text{ L}$ ( $3 \times 2 \text{ L}$ )	$18 \text{ L}$ ( $3 \times 3 \times 2 \text{ L}$ )
Temperature	Directly	Clean HDPE bottle		
TSS	Directly	Clean HDPE bottle		
POC	Directly	Clean glass bottle		



Number of samples, sampling time and sampling location is shown in Table 2.

**Table 2. Sampling Times**

No.	Sampling point	Sample Number	Sampling site	Sampling Date	Sampling Time
013	S1	12	The east China sea near the yellow sea	2013.09.15	12:10-13:00
	S2	12			13:10-14:00
	S3	12	The Northern yellow sea	2013.09.17	08:10-09:00
	S4	36			09:10-10:00
014	S1	12	South of the yellow sea	2013.10.11	10:20-11:00
	S2	12			16:00-16:40
	S3	12	The Bohai Sea	2013.10.13	15:10-16:00
	S4	36			16:25-17:15
015	S1	12	The east China sea near the yellow sea	2013.10.27	12:00-12:50
	S2	12			13:05-13:55
	S3	12	The central yellow sea	2013.10.29	11:30-12:20
	S4	36			12:35-13:25
016	S1	12	The central yellow sea	2013.10.29	13:40-14:30
	S2	12			14:45-15:35
	S3	12	The Bohai Sea	2013.10.31	11:35-12:25
	S4	36			12:40-13:30
017	S1	12	The southern yellow sea	2014.04.03	13:10-14:00
	S2	12			14:10-15:00
	S3	12	The Bohai Sea	2014.04.05	08:05-08:45
	S4	36			09:05-09:55

### 3.2.2 Sample preservation and transportation

After collecting samples of organisms  $\geq 10\text{-}50\text{ }\mu\text{m}$ , added algae staining solution of 2 mL into the sediment drum for 15 minutes, and then added formalin to fix the sample. After collecting sample of organisms  $\geq 50\text{ }\mu\text{m}$ , added five drops of staining solution for 30 min, and then added formalin to fix the sample. The bacteria samples are processed immediately after sampling on board and cooled at  $4^{\circ}\text{C}$  until analyze.

Parameters like salinity and temperature are measured in situ immediately after sampling.

TSS and POC should be processed within 24 h after sampling and stored in dark. Samples are packed in a cooler bag ( $4^{\circ}\text{C}$ ) under transport. When the samples arrived at the laboratory, they are stored in a cool room. If POC testing cannot be finished within 24 h, add a small amount of  $\text{HgCl}_2$  in sample and stored under  $-20^{\circ}\text{C}$  for 7 days.

Preservation methods and expected storage/holding times before measurement are shown in Table 3.

**Table 3. Preservation methods and expected storage/holding times before measurement**

Parameter	Processing/Preservation	Max. holding time	Expected storage time
Organisms $\geq 50$ $\mu\text{m}$	Stain with janus green B, and add formalin.	6 months	<6 months
Organisms $\geq 10$ -50 $\mu\text{m}$	Stain with algae staining solution, and add formalin for preserving sample.	6 months	<6 months
Heterotrophic bacteria <i>E. coli</i> , Intestinal <i>Enterococci</i> , <i>Vibrio cholerae</i>	Process in-situ and store at 4°C until analyze. Enumerate using appropriate media.	6 hours	<6 hours
Salinity	Analyze immediately using salimeter in-situ.	-	0
Temperature	Analyze immediately using thermometer in-situ.	-	0
TSS	Filter immediately after sampling in-situ. Store filter membrane at 4°C.	2 months	<2 months
POC	Store in the dark until processing. Add $\text{HgCl}_2$ and store under -20°C.	7 days	0-5 days

### 3.3 Analytical Methods

#### 3.3.1 Water qualities analysis

##### 3.3.1.1 Temperature

Temperature is measured *in situ* using a calibrated thermometer. Temperature is reported in °C.





### 3.3.1.2 Salinity

Salinity is measured *in situ* using a calibrated salimeter. Salinity is reported in PSU.



### 3.3.1.3 Particulate Organic Carbon (POC)

POC is determined based on spectrophotometry method. According to “Chinese Gulf ecosystem observation method (2005)”, carbon is wet oxidized by acidic dichromate; the decrease of the extinction value of the yellow dichromate solution may indicate the quantum of oxidized carbon.

### 3.3.1.4 Total Suspended Solids (TSS)

The sample is filtered through a filter (0.45  $\mu\text{m}$ ) on board, and the filtered membrane is stored at 4°C until analysis. TSS is measured at BWDL in accordance to National Standard of China (2007): The Specification for Marine Monitoring (GB 17378.4) - Part 4: Seawater analysis.



## 3.3.2 Viable organism analysis

### *Determination and quantification of organisms $\geq 50 \mu\text{m}$*

The filtered and concentrated samples were identified and analyzed by total count method and counted by kind/species to calculate the organism number (number of organisms in per unit)



(GB17378.7-2007):

$$r_B = \frac{N_B}{V}$$

Where:

$r_B$ ——density of organisms  $\geq 50 \mu\text{m}$  in per unit of volume;

$N_B$ ——number of zooplanktons, ind.;

$V$ ——volume of filtered water,  $\text{m}^3$ .

***Determination and quantification of organisms  $\geq 10\text{-}50 \mu\text{m}$***

Lightly absorb the supernatant fluid from the pretreated samples using a suction pipe with  $10 \mu\text{m}$  bolting silk. After settling down for a few times, the water sample was condensed to a  $50 \text{ mL}$  thimble tube. Shake enough before sampling counting, absorb a certain amount of sample and then release it at the counting chamber covered with cover glass (make sure there are no bubbles remain) and then conduct the microscopic counting (GB17378.7-2007).

Optical microscopic counting (concentrated counting):

$$C = \frac{n \times V_1}{V_2 \times V_n}$$

Where:

$C$ ——total amount of samples in per unit volume, cells/ $\text{mL}$ ;

$n$ ——number of samples, cells;

$V_1$ ——the volume of concentrated water sample,  $\text{mL}$ ;

$V_2$ ——the volume of filtered water,  $\text{mL}$ ;

$V_n$ ——the count volume of water sample,  $\text{mL}$ .



### ***Bacteria***

#### ***Heterotrophic bacteria***

Heterotrophic bacteria were quantified according to a modified version of the specification for marine monitoring--Part 7: Ecological survey of offshore pollution and biological monitoring GB17378.7-2007/10.1 using a marine agar for isolation of marine heterotrophic bacteria.

#### ***E.coli***

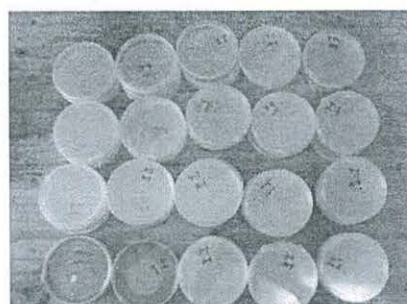
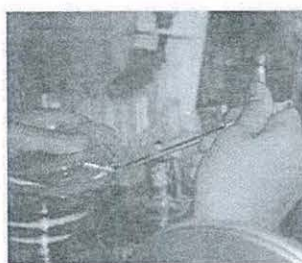
*E. coli* were quantified according to the specification for marine monitoring--Part 7: Ecological survey of offshore pollution and biological monitoring GB17378.7-2007/9.1 or drinking water standard test methods microbial indicators 5750.12-2006/4.1 at a temperature of  $36\pm 1^{\circ}\text{C}$  and over an incubation period of 18-24 hours.

#### ***Enterococcus group***

Total fecal *Enterococci* were quantified according to detection of *Enterococci* in water - Part 2: ISO 7899-2:2000 at a temperature of  $36\pm 2^{\circ}\text{C}$  and over an incubation period of 44 hours.

### *Vibrio cholera*

According to “Diagnosis Standard for *Vibrio cholerae* (WS 289-2008)”, water samples are inoculated in basic peptone water medium. Place the enrichment medium with samples into incubator for enlargement cultivation at  $37^{\circ}\text{C}$  for 6-8 h. Then the strong and weak nutrient mediums are inoculated, they are used to isolated culture and made cultivated substance morphology observation. *Vibrio cholera* in different isolation mediums presents different characteristics. Identify the typical colonies appeared in the strong and weak nutrient mediums via slide agglutination test and oxidase test.





## 4. Results and discussion

### 4.1 Physical and chemical water qualities

Table 4 shows temperature, salinity, TTS and POC of influent water and treated water (i.e., treated by Marine<sup>TM</sup> BWMS) for five test cycles during the shipboard testing conducted from 2013 September 15 to 2014 April 5.

#### 4.1.1 Temperature and Salinity

The influent water of test No. 013 were collected from the East China Sea near the Yellow Sea, and the treated water were discharged into the Northern Yellow Sea. The average water temperature and salinity of influent ballast water in the East China Sea near the Yellow Sea, were 25.5 °C and 30.8 PSU, respectively.

The influent water of test No. 014 were collected from the Southern Yellow Sea, and the treated water were discharged into the Bohai Sea. The average water temperature and salinity of influent ballast water were 22.5 °C and 29.8 PSU, respectively.

The influent water of test No. 015 were collected from the East China Sea near the Yellow Sea, and the treated water were discharged into the central Yellow Sea. The average water temperature and salinity of influent ballast water were 25.3 °C and 31.2 PSU, respectively.

The influent water of test No. 016 were collected from the central Yellow Sea, and the treated water were discharged into the Bohai Sea. The average water temperature and salinity of influent ballast water were 22.2 °C and 29.5 PSU, respectively.

The influent water of test No. 017 were collected from the Southern Yellow Sea, and the treated water were discharged into the Bohai Sea. The average water temperature and salinity of influent ballast water were 12.0 °C and 31.1 PSU, respectively (Table 4).

#### 4.1.2 TSS and POC

TSS levels of influent water at uptake in test No. 013, 015 and 016 were larger than 50 mg/L, among which test No. 016 was the highest. TSS levels of influent water at uptake in test No. 014 and 017 at the level of 22.3 and 24.0 mg/L were relatively lower than those of test No. 013, 015 and 016. Generally TSS levels of treated water decreased significantly due to filtration.

It was observed that the concentrations of POC in influent water varied within 0.13-0.32 mg/L. The concentrations of POC in discharged water in test No. 016 and 017 decreased slightly compared to those in influent water; the concentrations of POC in test No. 013 and 015 had not a significant change; however, the concentrations of POC in discharged water in test No. 014 were higher than that in influent water.

**Table 4. Physical and chemical water qualities. Mean  $\pm$  standard deviation of replicate measurements (n=3)**

<b>Test No. 013</b>				
Uptake from the East China Sea near the Yellow Sea (2013.09.15), and discharge to the Northern Yellow Sea (2013.09.17)				
sampling point	Temperature (°C)	Salinity (PSU)	TSS (mg/L)	POC (mg/L)
S1	25.5 $\pm$ 0.5	30.8 $\pm$ 0.9	54.1 $\pm$ 3.0	0.31 $\pm$ 0.06
S4	23.6 $\pm$ 0.1	30.2 $\pm$ 0.1	46.9 $\pm$ 9.0	0.33 $\pm$ 0.07
S2	24.5 $\pm$ 0.2	31.2 $\pm$ 0.1	60.0 $\pm$ 0.1	0.30 $\pm$ 0.06
S3	22.6 $\pm$ 0.2	31.0 $\pm$ 0.1	41.3 $\pm$ 3.9	0.30 $\pm$ 0.07
<b>Test No. 014</b>				
Uptake from the Southern Yellow Sea (2013.10.11), and discharge to the Bohai Sea (2013.10.13)				
sampling point	Temperature (°C)	Salinity (PSU)	TSS (mg/L)	POC (mg/L)
S1	22.5 $\pm$ 0.1	29.8 $\pm$ 0.0	22.3 $\pm$ 1.0	0.13 $\pm$ 0.08
S4	21.4 $\pm$ 0.1	29.6 $\pm$ 0.1	52.4 $\pm$ 4.7	0.31 $\pm$ 0.10
S2	22.5 $\pm$ 0.1	29.8 $\pm$ 0.0	23.6 $\pm$ 0.3	0.19 $\pm$ 0.08
S3	21.6 $\pm$ 0.0	29.7 $\pm$ 0.0	46.9 $\pm$ 1.8	0.18 $\pm$ 0.03
<b>Test No. 015</b>				
Uptake from the East China Sea near the Yellow Sea (2013.10.27), and discharge to the central Yellow Sea (2013.10.29)				
sampling point	Temperature (°C)	Salinity (PSU)	TSS (mg/L)	POC (mg/L)
S1	25.3 $\pm$ 0.1	31.2 $\pm$ 0.1	58.2 $\pm$ 3.5	0.31 $\pm$ 0.10
S4	24.1 $\pm$ 0.5	31.1 $\pm$ 0.1	27.4 $\pm$ 4.0	0.34 $\pm$ 0.10
S2	25.2 $\pm$ 0.1	31.1 $\pm$ 0.1	62.8 $\pm$ 4.2	0.30 $\pm$ 0.08

S3	23.9±1.1	31.1±0.1	33.2±5.3	0.28±0.07
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**Test No. 016**

Uptake from the Yellow Sea (2013.10.29), and discharge to the Bohai Sea (2013.10.31)

sampling point	Temperature (°C)	Salinity (PSU)	TSS (mg/L)	POC (mg/L)
S1	22.2±0.1	29.5±0.3	64.5±2.3	0.27±0.03
S4	21.4±0.2	29.8±0.1	32.4±4.1	0.14±0.06
S2	22.2±0.1	29.7±0.0	61.2±6.0	0.37±0.05
S3	21.4±0.1	29.8±0.2	54.4±2.6	0.22±0.04

**Test No. 017**

Uptake from the Southern Yellow Sea (2014.04.03), and discharge to the Bohai Sea (2014.04.03)

sampling point	Temperature (°C)	Salinity (PSU)	TSS (mg/L)	POC (mg/L)
S1	12.0±0.0	31.1±0.1	24.0±3.8	0.32±0.20
S4	11.8±0.2	31.1±0.1	23.8±9.9	0.15±0.03
S2	12.0±0.1	31.1±0.1	21.5±1.7	0.29±0.10
S3	11.7±0.1	31.2±0.1	20.1±0.4	0.16±0.00

**4.2 Organisms in treated water at discharge****4.2.1 Determination and quantification of organisms  $\geq 50 \mu\text{m}$** 

Table 5 shows the test results of living organisms  $\geq 50 \mu\text{m}$  during for five test cycles during the shipboard testing conducted from 2013 September 15 to 2014 April 5.

Among the five test cycles, 3 categories and 10 species of organisms  $\geq 50 \mu\text{m}$  were detected in influent water in test No. 013 in the East China Sea near the Yellow Sea with an average density of  $2.44 \times 10^3 \text{ ind./m}^3$ . 3 categories and 5 species of organisms  $\geq 50 \mu\text{m}$  were detected in influent water in test No. 014 in the Southern Yellow Sea with an average density of  $2.32 \times 10^3 \text{ ind./m}^3$ . 2 categories and 6 species of organisms  $\geq 50 \mu\text{m}$  were detected in influent water in test No. 015 in the East China Sea near the Yellow Sea with an average density of  $1.82 \times 10^4 \text{ ind./m}^3$ . 3 categories and 10 species of organisms  $\geq 50 \mu\text{m}$  were detected in influent water in test No. 016 in the central Yellow Sea with an average density of  $7.22 \times 10^3 \text{ ind./m}^3$ . 4 categories and 10 species of organisms  $\geq 50 \mu\text{m}$  were detected in influent water of in test No.



017 in the central Yellow Sea with an average density of  $6.44 \times 10^3$  ind./m<sup>3</sup>. The densities of organisms  $\geq 50 \mu\text{m}$  for the five test cycles met the IMO G8 requirements.

**Table 5. The average density of living organisms  $\geq 50 \mu\text{m}$**

<b>Test No. 013</b>				
Uptake from the East China Sea near the Yellow Sea (2013.09.15), and discharge to the Northern Yellow Sea (2013.09.17)				
	Treatment		Control	
	S1	S4	S2	S3
	(n=3)	(n=9)	(n=3)	(n=3)
Density				
(ind./m <sup>3</sup> )	$(2.44 \pm 0.24) \times 10^3$	$(0.45 \pm 0.31) \times 10^2$	$(4.19 \pm 1.30) \times 10^3$	$(1.47 \pm 0.15) \times 10^3$
<b>Test No. 014</b>				
Uptake from the Southern Yellow Sea (2013.10.11), and discharge to the Bohai Sea (2013.10.13)				
	Treatment		Control	
	S1	S4	S2	S3
	(n=3)	(n=9)	(n=3)	(n=3)
Density				
(ind./m <sup>3</sup> )	$(2.32 \pm 0.20) \times 10^3$	$6.67 \pm 1.94$	$(2.13 \pm 0.016) \times 10^3$	$(2.00 \pm 0.17) \times 10^3$
<b>Test No. 015</b>				
Uptake from the East China Sea near the Yellow Sea (2013.10.27), and discharge to the central Yellow Sea (2013.10.29)				
	Treatment		Control	
	S1	S4	S2	S3
	(n=3)	(n=9)	(n=3)	(n=3)
Density				
(ind./m <sup>3</sup> )	$(1.82 \pm 0.047) \times 10^4$	$6.11 \pm 1.96$	$(3.33 \pm 0.24) \times 10^4$	$(1.09 \pm 0.05) \times 10^4$
<b>Test No. 016</b>				
Uptake from the Yellow Sea (2013.10.29), and discharge to the Bohai Sea (2013.10.31)				
	Treatment		Control	
	S1	S4	S2	S3
	(n=3)	(n=9)	(n=3)	(n=3)
Density				
(ind./m <sup>3</sup> )	$(7.22 \pm 0.44) \times 10^3$	$1.89 \pm 1.27$	$(6.77 \pm 0.28) \times 10^3$	$(6.88 \pm 0.31) \times 10^3$
<b>Test No. 017</b>				
Uptake from the Southern Yellow Sea (2014.04.03), and discharge to the Bohai Sea (2014.04.03)				

	Treatment		Control	
	S1	S4	S2	S3
	(n=3)	(n=9)	(n=3)	(n=3)
Density				
(ind./m <sup>3</sup> )	$(6.44 \pm 0.77) \times 10^3$	$3.22 \pm 1.56$	$(6.13 \pm 0.67) \times 10^3$	$(1.34 \pm 0.29) \times 10^3$

Except test No. 013, organisms  $\geq 50 \mu\text{m}$  in discharged water from treated tanks all met the D-2 discharge standard. And the organisms  $\geq 50 \mu\text{m}$  in discharged water from control tanks in the five test cycles met the IMO G8 requirements.

In test No. 014, the densities of organisms  $\geq 50 \mu\text{m}$  in discharged water from treated tanks were significantly decreased after treatment from  $2.32 \times 10^3$  to  $6.67 \text{ ind./m}^3$ , while the average densities of organism  $\geq 50 \mu\text{m}$  in discharged water from control tanks were  $2.00 \times 10^3 \text{ ind./m}^3$ .

In test No. 015, the densities of organisms  $\geq 50 \mu\text{m}$  in discharged water from treated tanks were significantly decreased after treatment from  $1.82 \times 10^4$  to  $6.11 \text{ ind./m}^3$ , while the average densities of organism  $\geq 50 \mu\text{m}$  in discharged water from control tanks were  $1.09 \times 10^4 \text{ ind./m}^3$ .

In test No. 016, the densities of organisms  $\geq 50 \mu\text{m}$  in discharged water from treated tanks were significantly decreased after treatment from  $7.22 \times 10^3$  to  $1.89 \text{ ind./m}^3$ , while the average densities of organism  $\geq 50 \mu\text{m}$  in discharged water from control tanks were  $6.88 \times 10^3 \text{ ind./m}^3$ .

In test No. 017, the densities of organisms  $\geq 50 \mu\text{m}$  in discharged water from treated tanks were significantly decreased after treatment from  $6.44 \times 10^3$  to  $3.22 \text{ ind./m}^3$ , while the average densities of organism  $\geq 50 \mu\text{m}$  in discharged water from control tanks were  $1.34 \times 10^3 \text{ ind./m}^3$ .

#### 4.2.2 Determination and quantification of organisms $\geq 10\text{-}50 \mu\text{m}$

Table 6 shows the test results of living organisms  $\geq 10\text{-}50 \mu\text{m}$  during for five test cycles during the shipboard testing conducted from 2013 September 15 to 2014 April 5.

Among the five test cycles, 3 categories and 8 species of organisms  $\geq 10\text{-}50 \mu\text{m}$  were detected in influent water in test No. 013 in the East China Sea near the Yellow Sea with an average density of  $5.05 \times 10^2 \text{ cells/mL}$ . 4 categories and 10 species of organisms  $\geq 10\text{-}50 \mu\text{m}$  were detected in influent water in test No. 014 in the Southern Yellow Sea with an average density of  $1.40 \times 10^2 \text{ cells/mL}$ . 1 category and 6 species of organisms  $\geq 10\text{-}50 \mu\text{m}$  were detected in influent water in test No. 015 in the East China Sea near the Yellow Sea with an average

density of  $4.29 \times 10^2$  cells/mL. 3 categories and 11 species of organisms  $\geq 10\text{-}50\text{ }\mu\text{m}$  were detected in influent water in test No. 016 in the central Yellow Sea with an average density of  $1.36 \times 10^2$  cells/mL. 1 category and 12 species of organisms  $\geq 10\text{-}50\text{ }\mu\text{m}$  were detected in influent water of in test No. 017 in the central Yellow Sea with an average density of  $3.25 \times 10^2$  cells/mL. The main species of organisms  $\geq 10\text{-}50\text{ }\mu\text{m}$  were diatoms. Densities of organisms  $\geq 10\text{-}50\text{ }\mu\text{m}$  for the five test cycles met the IMO G8 requirements.

The average densities of organisms  $\geq 10\text{-}50\text{ }\mu\text{m}$  in discharged water from control tanks in the five test cycles were larger than 10 cells/mL, which met the IMO G8 requirements.

In test No. 013, the densities of organisms  $\geq 10\text{-}50\text{ }\mu\text{m}$  in discharged water from treated tanks were significantly decreased after treatment from  $5.05 \times 10^2$  to 2.78 cells/mL, while the average densities of organism  $\geq 10\text{-}50\text{ }\mu\text{m}$  in discharged water from control tanks were  $3.28 \times 10^2$  cells/mL.

In test No. 014, the densities of organisms  $\geq 10\text{-}50\text{ }\mu\text{m}$  in discharged water from treated tanks were significantly decreased after treatment from  $1.40 \times 10^2$  to 0.29 cells/mL, while the average densities of organism  $\geq 10\text{-}50\text{ }\mu\text{m}$  in discharged water from control tanks were 12.67 cells/mL.

In test No. 015, the densities of organisms  $\geq 10\text{-}50\text{ }\mu\text{m}$  in discharged water from treated tanks were significantly decreased after treatment from  $4.29 \times 10^2$  to 0.04 cells/mL, while the average densities of organism  $\geq 10\text{-}50\text{ }\mu\text{m}$  in discharged water from control tanks were 43.33 cells/mL.

In test No. 016, the densities of organisms  $\geq 10\text{-}50\text{ }\mu\text{m}$  in discharged water from treated tanks were significantly decreased after treatment from  $1.36 \times 10^2$  to 0.02 cells/mL, while the average densities of organism  $\geq 10\text{-}50\text{ }\mu\text{m}$  in discharged water from control tanks were 15.00 cells/mL.

In test No. 017, the densities of organisms  $\geq 10\text{-}50\text{ }\mu\text{m}$  in discharged water from treated tanks were significantly decreased after treatment from  $3.25 \times 10^2$  to 0.09 cells/mL, while the average densities of organism  $\geq 10\text{-}50\text{ }\mu\text{m}$  in discharged water from control tanks were  $1.15 \times 10^2$  cells/mL.

Therefore, organisms  $\geq 10\text{-}50\text{ }\mu\text{m}$  in discharged water from treated tanks all met D-2 discharge



standard and organisms  $\geq 10\text{-}50\text{ }\mu\text{m}$  in discharged water from control tanks all met IMO G8 requirement.

**Table 6. The average density of living organisms  $\geq 10\text{-}50\text{ }\mu\text{m}$**

**Test No. 013**

Uptake from the East China Sea near the Yellow Sea (2013.09.15), and discharge to the Northern Yellow Sea (2013.09.17)

	Treatment		Control	
	S1	S4	S2	S3
	(n=3)	(n=9)	(n=3)	(n=3)
Density				
(cells/mL)	$(5.05 \pm 0.78) \times 10^2$	$2.78 \pm 2.64$	$(4.92 \pm 0.96) \times 10^2$	$(3.28 \pm 0.28) \times 10^2$

**Test No. 014**

Uptake from the Southern Yellow Sea (2013.10.11), and discharge to the Bohai Sea (2013.10.13)

	Treatment		Control	
	S1	S4	S2	S3
	(n=3)	(n=9)	(n=3)	(n=3)
Density				
(cells/mL)	$(1.40 \pm 0.22) \times 10^2$	$0.29 \pm 0.35$	$(1.33 \pm 0.20) \times 10^2$	$12.67 \pm 1.99$

**Test No. 015**

Uptake from the East China Sea near the Yellow Sea (2013.10.27), and discharge to the central Yellow Sea (2013.10.29)

	Treatment		Control	
	S1	S4	S2	S3
	(n=3)	(n=9)	(n=3)	(n=3)
Density				
(cells/mL)	$(4.29 \pm 1.19) \times 10^2$	$0.04 \pm 0.07$	$(3.97 \pm 0.80) \times 10^2$	$43.33 \pm 36.86$

**Test No. 016**

Uptake from the Yellow Sea (2013.10.29), and discharge to the Bohai Sea (2013.10.31)

	Treatment		Control	
	S1	S4	S2	S3
	(n=3)	(n=9)	(n=3)	(n=3)
Density				
(cells/mL)	$(1.36 \pm 0.24) \times 10^2$	$0.02 \pm 0.04$	$(1.58 \pm 0.15) \times 10^2$	$15.00 \pm 1.00$

**Test No. 017**

Uptake from the Southern Yellow Sea (2014.04.03), and discharge to the Bohai Sea (2014.04.03)

	Treatment	Control
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	S1 (n=3)	S4 (n=9)	S2 (n=3)	S3 (n=3)
Density (cells/mL)	$(3.25 \pm 0.82) \times 10^2$	$0.09 \pm 0.11$	$(2.25 \pm 1.00) \times 10^2$	$(1.15 \pm 0.18) \times 10^2$

### 4.2.3 Bacteria

Table 7 shows the test results of bacteria during for five test cycles during the shipboard testing conducted from 2013 September 15 to 2014 April 5.

Among the five test cycles, the densities of heterotrophic bacteria in the influent water in test No. 015 from the East China Sea near the Yellow Sea was largest, which was up to  $7.3 \times 10^4$  CFU/mL. In test No. 014 and 016, the densities of heterotrophic bacteria in the influent water were  $2.1 \times 10^4$  and  $2.7 \times 10^4$  CFU/mL, respectively. In test No. 013 and 017, the densities of heterotrophic bacteria in the influent water were lower, which were  $1.1 \times 10^4$  and  $1.0 \times 10^4$  CFU/mL, respectively.

The average densities of *E. coli* in the influent water in test No. 015 was highest with a level of  $4.3 \times 10^5$  CFU/100 mL, while the densities of *E. coli* in other four test cycles were  $1.8 \times 10^5$ ,  $2.9 \times 10^5$ ,  $1.4 \times 10^5$  and  $1.5 \times 10^4$  CFU/100 mL, respectively.

The test results showed that the concentrations of *E. coli* in discharged water from treated tanks decreased significantly in five test cycles, which were 116.1, 99.8, 61.7, 35.7 and 46.0 CFU/100 mL, respectively, whereas the average concentrations of *E. coli* in discharged water from control tanks were  $1.4 \times 10^5$ ,  $5.4 \times 10^4$ ,  $4.9 \times 10^5$ ,  $8.2 \times 10^4$  and  $6.4 \times 10^3$  CFU/100 mL, respectively. The above test results met the IMO G8 requirement and D-2 discharge standard.

Intestinal *enterococci* were detected in test No. 013 and 017, while test No. 014, 015 and 016 were not. No intestinal *enterococci* were detected in discharged water either from treated tanks or control tanks in five test cycles. No *vibrio cholerae* were detected in any samples.

Table 7. Test results of bacteria

<b>Test No. 013</b>				
Uptake from the East China Sea near the Yellow Sea (2013.09.15), and discharge to the Northern Yellow Sea (2013.09.17)				
	Sampling points			
	S1	S4	S2	S3
	(n=3)	(n=9)	(n=3)	(n=3)
Heterotrophic Bacteria (CFU/mL)	$(1.1 \pm 0.1) \times 10^4$	$333.9 \pm 263.4$	$(4.7 \pm 5.5) \times 10^4$	$(2.4 \pm 1.4) \times 10^4$
<i>E. coli</i> (CFU/100 mL)	$(1.8 \pm 0.7) \times 10^5$	$116.1 \pm 109.7$	$(1.6 \pm 0.5) \times 10^5$	$(1.4 \pm 1.9) \times 10^5$
intestinal <i>enterococci</i> (CFU/100 mL)	ND	ND	<2	ND
<i>vibrio cholerae</i> (CFU/100 mL)	ND	ND	ND	ND
<b>Test No. 014</b>				
Uptake from the Southern Yellow Sea (2013.10.11), and discharge to the Bohai Sea (2013.10.13)				
	Sampling points			
	S1	S4	S2	S3
	(n=3)	(n=9)	(n=3)	(n=3)
Heterotrophic Bacteria (CFU/mL)	$(2.1 \pm 0.5) \times 10^4$	$297.7 \pm 186.4$	$(1.9 \pm 0.4) \times 10^4$	$(4.6 \pm 4.1) \times 10^4$
<i>E. coli</i> (CFU/100 mL)	$(2.9 \pm 2.2) \times 10^5$	$99.8 \pm 82.0$	$(1.4 \pm 1.5) \times 10^5$	$(5.4 \pm 1.3) \times 10^4$
intestinal <i>enterococci</i> (CFU/100 mL)	ND	ND	ND	ND
<i>vibrio cholerae</i> (CFU/100 mL)	ND	ND	ND	ND
<b>Test No. 015</b>				
Uptake from the East China Sea near the Yellow Sea (2013.10.27), and discharge to the central Yellow Sea (2013.10.29)				
	Sampling points			
	S1	S4	S2	S3
	(n=3)	(n=9)	(n=3)	(n=3)
Heterotrophic Bacteria (CFU/mL)	$(7.3 \pm 5.7) \times 10^4$	$271.8 \pm 261.4$	$(3.9 \pm 2.8) \times 10^4$	$(3.8 \pm 4.6) \times 10^4$
<i>E. coli</i> (CFU/100 mL)	$(4.3 \pm 3.4) \times 10^5$	$61.7 \pm 49.8$	$(2.1 \pm 1.2) \times 10^5$	$(4.9 \pm 4.1) \times 10^5$
intestinal <i>enterococci</i> (CFU/100 mL)	ND	ND	ND	ND
<i>vibrio cholerae</i> (CFU/100 mL)	ND	ND	ND	ND

Test No. 016



## Uptake from the Yellow Sea (2013.10.29), and discharge to the Bohai Sea (2013.10.31)

	Sampling points			
	S1	S4	S2	S3
	(n=3)	(n=9)	(n=3)	(n=3)
Heterotrophic Bacteria (CFU/mL)	$(2.7 \pm 0.6) \times 10^4$	527.8 $\pm$ 446.2	$(7.5 \pm 10.0) \times 10^4$	$(6.5 \pm 1.3) \times 10^4$
<i>E. coli</i> (CFU/100 mL)	$(1.4 \pm 1.4) \times 10^5$	35.7 $\pm$ 19.1	$(1.2 \pm 0.3) \times 10^5$	$(8.2 \pm 1.6) \times 10^4$
intestinal <i>enterococci</i> (CFU/100 mL)	ND	ND	ND	ND
<i>vibrio cholerae</i> (CFU/100 mL)	ND	ND	ND	ND

## Test No. 017

## Uptake from the Southern Yellow Sea (2014.04.03), and discharge to the Bohai Sea (2014.04.03)

	Sampling points			
	S1	S4	S2	S3
	(n=3)	(n=9)	(n=3)	(n=3)
Heterotrophic Bacteria (CFU/mL)	$(1.0 \pm 0.2) \times 10^4$	85.7 $\pm$ 35.5	$(1.1 \pm 0.4) \times 10^4$	$(6.5 \pm 0.9) \times 10^3$
<i>E. coli</i> (CFU/100 mL)	$(1.5 \pm 0.7) \times 10^4$	46.0 $\pm$ 36.8	$(7.9 \pm 1.6) \times 10^3$	$(6.4 \pm 1.3) \times 10^3$
intestinal <i>enterococci</i> (CFU/100 mL)	<0.3	ND	<0.3	ND
<i>vibrio cholerae</i> (CFU/100 mL)	ND	ND	ND	ND

ND: not detected.

## 5. Conclusions

203-day shipboard tests for five batches were conducted on "Hongtai 158", using PACT Marine<sup>TM</sup> Ballast Water Management System (BWMS) (with a capacity of 300 m<sup>3</sup>/h), developed by Yixing PACT Environmental Protection Technology Co., Ltd. The test periods met requirement of IMO G8 Guidelines. The test results were compared to IMO G8 requirement and D-2 standard and the following conclusions were drawn:

1. For test No. 013, densities of organisms  $\geq 50 \mu\text{m}$  in discharged water of PACT Marine<sup>TM</sup> BWMS (with a capacity of 300 m<sup>3</sup>/h) were higher than the requirement of IMO G8, so it was an invalid cycle. Densities of organisms  $\geq 50 \mu\text{m}$  in influent water for the subsequent four cycle tests (i.e., test No. 014-017) were all within  $2.32 \times 10^3$ - $1.82 \times 10^4$  ind./m<sup>3</sup> with average density of  $8.55 \times 10^3$  ind./m<sup>3</sup>, which met the IMO G8 requirement. Average densities of organisms  $\geq 50 \mu\text{m}$  in discharged water from treated tanks for four valid tests (i.e., test No. 014-017), were 6.67, 6.11, 1.89 and 3.22 ind./m<sup>3</sup>, respectively. Average densities of organisms  $\geq 50 \mu\text{m}$  in discharged water from control tanks for four valid tests (i.e., test No. 014-017), were  $2.00 \times 10^3$ ,  $1.09 \times 10^4$ ,  $6.88 \times 10^3$  and  $1.34 \times 10^3$  ind./m<sup>3</sup>, respectively. Densities of organisms  $\geq 50 \mu\text{m}$  in discharged water both from treated tanks and control tanks were met D-2 discharge standard and IMO G8 requirement.
2. Densities of organisms  $\geq 10$ - $50 \mu\text{m}$  in influent water for five test cycles were all within  $1.36 \times 10^2$ - $5.05 \times 10^2$  cells/mL with an average density of  $3.07 \times 10^2$  cells/mL, which met the requirement of IMO G8. Average densities of living organisms  $\geq 10$ - $50 \mu\text{m}$  in discharged water from treated tanks were 2.78, 0.29, 0.04, 0.02 and 0.09 cells/mL, respectively. Average densities of living organisms  $\geq 10$ - $50 \mu\text{m}$  in discharge water from control tanks were  $3.28 \times 10^2$ , 12.67, 43.33, 15.00 and  $1.15 \times 10^2$  cells/mL, respectively. Organisms  $\geq 10$ - $50 \mu\text{m}$  in discharged water from treated tanks and control tanks met D-2 discharge standard and IMO G8 requirement, respectively.
3. Total number of heterotrophic bacteria in influent water for the five tests (i.e., test No. 013-017) were  $1.1 \times 10^4$ ,  $2.1 \times 10^4$ ,  $7.3 \times 10^4$ ,  $2.7 \times 10^4$  and  $1.0 \times 10^4$  CFU/mL, respectively. Average concentrations of heterotrophic bacteria in discharged water from treated tank for the five tests (i.e., test No. 013-017) were 333.9, 297.7, 271.8, 527.8 and 85.7 CFU/mL, respectively. The average concentrations of heterotrophic bacteria in discharged water from control tanks were  $2.4 \times 10^4$ ,  $4.6 \times 10^4$ ,  $3.8 \times 10^4$ ,  $6.5 \times 10^4$  and  $6.5 \times 10^3$  CFU/mL, respectively.

4. Average concentrations of *Escherichia coli* in influent water for the five tests (i.e., test No. 013-017) were within  $1.4 \times 10^4$ - $4.3 \times 10^5$  CFU/100 mL. Average concentrations of *Escherichia coli* in discharged water from treated tanks for the five tests (i.e., test No. 013-017) were 116.1, 99.8, 61.7, 35.7 and 46.0 CFU/100 mL, respectively. The average concentrations of *Escherichia coli* in discharge water from control tanks were  $1.4 \times 10^5$ ,  $5.4 \times 10^4$ ,  $4.9 \times 10^5$ ,  $8.2 \times 10^4$  and  $6.4 \times 10^3$  CFU/100 mL, respectively. These results met IMO G8 requirement and D-2 discharge standard.

5. No intestinal *enterococci* and *vibrio cholerae* were detected in discharged water for the five cycle tests (i.e., test No. 013-017).

In summary, except test No. 013, the other four cycle tests (i.e., test No. 014-017) were consecutive and effective, and their test procedures and results met IMO G8 requirement and D-2 standard.

Note: The reason for unqualified density of organisms  $\geq 50 \mu\text{m}$  in discharged water of test No. 013 was attributed to the fact that the ballast tank on Hongtai 158 was not cleaned up before the installation of PACT Marine<sup>TM</sup> BWMS (with a capacity of  $300 \text{ m}^3/\text{h}$ ).



Table 8. Comparison with D-2 standard and IMO G8 requirements

Cycles	Parameters	D-2 standard /IMO G8		Estimated Value			Remarks
		Uptake	Discharge	Uptake	Discharged water on the treatment track	Discharged water on the control track	
013	$\geq 50\mu\text{m}$ (ind./m <sup>3</sup> )	> 100	< 10	$2.44 \times 10^3$	45.11	$1.47 \times 10^3$	Not meet the D-2 and G8 requirements
	10~50 $\mu\text{m}$ (cells/mL)	> 100	< 10	$5.05 \times 10^2$	2.78	$3.28 \times 10^2$	Meet the D-2 and G8 requirements
	Heterotrophic Bacteria (CFU/mL)	> 10 <sup>4</sup>	N/A	$1.1 \times 10^4$	$3.3 \times 10^2$	$2.4 \times 10^4$	Meet the D-2 and G8 requirements
	<i>Escherichia Coli</i> (CFU/100mL)	N/A	< 250	$1.8 \times 10^5$	$1.2 \times 10^2$	$1.4 \times 10^5$	Meet the D-2 and G8 requirements
	<i>Enterococcus</i> (CFU/100mL)	N/A	< 100	ND	ND	ND	Meet the D-2 and G8 requirements
	<i>Vibrio cholerae</i> (CFU/100mL)	N/A	< 1	ND	ND	ND	Meet the D-2 and G8 requirements
014	$\geq 50\mu\text{m}$ (ind./m <sup>3</sup> )	> 100	< 10	$2.32 \times 10^3$	6.67	$2.00 \times 10^3$	Meet the D-2 and G8 requirements
	10~50 $\mu\text{m}$ (cells/mL)	> 100	< 10	$1.40 \times 10^2$	0.29	12.67	Meet the D-2 and G8 requirements
	Heterotrophic Bacteria (CFU/mL)	> 10 <sup>4</sup>	N/A	$2.1 \times 10^4$	$3.0 \times 10^2$	$4.6 \times 10^4$	Meet the D-2 and G8 requirements
	<i>Escherichia Coli</i> (CFU/100mL)	N/A	< 250	$2.9 \times 10^5$	99.8	$5.4 \times 10^4$	Meet the D-2 and G8 requirements
	<i>Enterococcus</i> (CFU/100mL)	N/A	< 100	ND	ND	ND	Meet the D-2 and G8 requirements
	<i>Vibrio cholerae</i> (CFU/100mL)	N/A	< 1	ND	ND	ND	Meet the D-2 and G8 requirements

Cycles	Parameters	D-2 standard /IMO G8		Estimated Value			Remarks
		Uptake	Discharge	Uptake	Discharged water on the treatment track	Discharged water on the control track	
015	$\geq 50\mu\text{m}$ (ind./m <sup>3</sup> )	> 100	< 10	$1.82 \times 10^4$	6.11	$1.09 \times 10^4$	Meet the D-2 and G8 requirements
	10~50 $\mu\text{m}$ (cells/mL)	> 100	< 10	$4.29 \times 10^2$	0.04	43.33	Meet the D-2 and G8 requirements
	Heterotrophic Bacteria (CFU/mL)	> $10^4$	N/A	$7.3 \times 10^4$	$2.7 \times 10^2$	$3.8 \times 10^4$	Meet the D-2 and G8 requirements
	<i>Escherichia Coli</i> (CFU/100mL)	N/A	< 250	$4.3 \times 10^5$	61.7	$4.9 \times 10^5$	Meet the D-2 and G8 requirements
	<i>Enterococcus</i> (CFU/100mL)	N/A	< 100	ND	ND	ND	Meet the D-2 and G8 requirements
	<i>Vibrio cholerae</i> (CFU/100mL)	N/A	< 1	ND	ND	ND	Meet the D-2 and G8 requirements
016	$\geq 50\mu\text{m}$ (ind./m <sup>3</sup> )	> 100	< 10	$7.22 \times 10^3$	1.89	$6.88 \times 10^3$	Meet the D-2 and G8 requirements
	10~50 $\mu\text{m}$ (cells/mL)	> 100	< 10	$1.36 \times 10^2$	0.02	15.00	Meet the D-2 and G8 requirements
	Heterotrophic Bacteria (CFU/mL)	> $10^4$	N/A	$2.7 \times 10^4$	$5.3 \times 10^2$	$6.5 \times 10^4$	Meet the D-2 and G8 requirements
	<i>Escherichia Coli</i> (CFU/100mL)	N/A	< 250	$1.4 \times 10^5$	35.7	$8.2 \times 10^4$	Meet the D-2 and G8 requirements
	<i>Enterococcus</i> (CFU/100mL)	N/A	< 100	ND	ND	ND	Meet the D-2 and G8 requirements
	<i>Vibrio cholerae</i> (CFU/100mL)	N/A	< 1	ND	ND	ND	Meet the D-2 and G8 requirements

Cycles	Parameters	D-2 standard /IMO G8		Estimated Value			Remarks
		Uptake	Discharge	Uptake	Discharged water on the treatment track	Discharged water on the control track	
017	$\geq 50\mu\text{m}$ (ind./m <sup>3</sup> )	> 100	< 10	$6.44 \times 10^3$	3.22	$1.34 \times 10^3$	Meet the D-2 and G8 requirements
	10~50 $\mu\text{m}$ (cells/mL)	> 100	< 10	$3.25 \times 10^2$	0.09	$1.15 \times 10^2$	Meet the D-2 and G8 requirements
	Heterotrophic Bacteria (CFU/mL)	> 10 <sup>4</sup>	N/A	$1.0 \times 10^4$	85.7	$6.5 \times 10^3$	Meet the D-2 and G8 requirements
	<i>Escherichia Coli</i> (CFU/100mL)	N/A	< 250	$1.5 \times 10^4$	46.0	$6.4 \times 10^3$	Meet the D-2 and G8 requirements
	<i>Enterococcus</i> (CFU/100mL)	N/A	< 100	< 0.3	ND	ND	Meet the D-2 and G8 requirements
	<i>Vibrio cholerae</i> (CFU/100mL)	N/A	< 1	ND	ND	ND	Meet the D-2 and G8 requirements

N/A means there is no requirement about this part. ND means there is not detected about this part.



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